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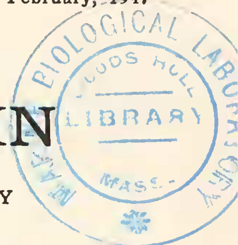

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A QUANTITATIVE STUDY OF PHENOCOPY PRODUCTION WITH MONOCHROMATIC ULTRAVIOLET IRRADIATION

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The term phenocopy was introduced by Goldschmidt (1935) to refer to forms, produced by some experimental procedure, whose appearance duplicates or copies the phenotype of some mutant or combination of mutants. The first experiments with phenocopy production were those of Standfuss (1896) who, by treating butterfly pupae with high or low temperatures, produced adults which resembled other geographic races. Phenocopies have been produced in Drosophila by high temperature (Goldschmidt, 1929, 1935; Plough and Ives, 1932, 1935; Child, Blanc, and Plough, 1940), low temperature (Gottschewski, 1934), X-rays (Friesen, 1936; Waddington, 1942; Villee, 1946a), chemical agents (Rapoport, 1939), visible light (Villee and Lavin, 1946), and ultraviolet light (Geigy, 1931; Eloff, 1939; Epsteins, 1939). Geigy irradiated only very early egg stages and obtained flies with abnormal abdomens, legs, and wings. Eloff was interested primarily in the effects of ultraviolet light on crossing-over but observed some wing abnormalities when late pupae were irradiated. Epsteins irradiated larvae and pupae and produced abnormal abdomen, hemithorax, the absence of the scutellum, and abnormalities in the wings, chiefly scalloping of the distal and posterior edges of the wings. In none of these experiments was the intensity of the ultraviolet light measured.

This study was undertaken to provide quantitative data of the effects of ultraviolet light on larval and pupal stages. It was originally planned to use both 2537 A and 2800 A light to see if the phenocopy-producing reactions could be ascribed to changes in nucleic acid or protein metabolism. However, a light source providing 2800 A light could not be obtained so experiments with that have been postponed. Davis (1944) made a quantitative study of the effects of ultraviolet in inhibiting the folding process in neural tube formation in chicks. By using monochromatic light of different wave-lengths obtained from a monochromator he determined the photochemical efficiency curve for the process. This was found to compare closely with the absorption curve of sterols, especially that of 7-dehydro-

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cholesterol, with maxima at 2576 A and 2804 A. Schechtman (1944) found that the inhibiting effects of ultraviolet light on the development of Hyla eggs were slightly stronger at 2537 A than at other bands tested and Landen and Uber (1939) found that the inactivation of yeast by ultraviolet was greatest at 2600 A, where 500 ergs/mm.[2] produced 50 per cent inactivation. Stadler and Uber (1942) found that the photochemical efficiency curve of ultraviolet light in producing mutations in maize corresponded to the absorption curve of nucleic acids and Hollaender (1945) and colleagues found that ultraviolet of wave-length 2600 A was much more efficient than other wave-lengths in producing mutations in a variety of fungi, Neurospora, Trichophyton, Penicillium, and Aspergillus.

Materials and Methods

Three stocks were irradiated: a wild type and an aristapedia-Bridges (ss^{aB}, chromosome 3, locus 58.8) stock isogenic with it (Villee, 1946b) and an independent, miniature wing (m, chromosome 1, locus 36.1) stock. Larvae were obtained by allowing large numbers of stock flies to lay eggs for a two-hour period on corn meal-molasses-agar food in half-pint bottles. The ages of the larvae are thus known to within $\pm$ one hour. The cultures were kept at 25.5° C. before and after irradiation. At this temperature the larvae pupate within a few hours of 100 hours after they hatch from the eggs. Prepupae (white pupae) were collected frequently and the time noted. The age of the pupae at irradiation was determined from this. A total of 3,500 flies was irradiated in groups of 25 larvae or pupae.

The source of the ultraviolet radiation was a spiral quartz mercury resonance lamp, manufactured by the Hanovia Chemical Company, which emits about 80 per cent of its energy in the form of the 2537 A line. A 120 mA. luminous tube transformer was used. The visible radiation was removed by a quartz filter cell containing a mixture of nickel sulfate and cobalt sulfate dissolved in distilled water (Backström, 1940). The lamp used had been burned well over 100 hours before the experiments began so the amount of radiation of wave-lengths shorter than 2537 A should be negligible. The intensity of the irradiation was varied by altering the distance between the lamp and the target. The intensity was measured by a Hanovia ultraviolet meter, the target of which was fastened to a carriage on an optical bench. The larvae or pupae to be irradiated were placed in a small uncovered Petri dish on this carriage. By moving the carriage back and forth, intensity measurements were taken before and after each irradiation and always checked very closely.

The larvae to be irradiated were removed from the culture bottles, washed briefly in 70 per cent alcohol, rinsed in Ringer's solution, dried on filter paper and placed in small, uncovered, dry Petri dishes. The larvae very shortly became stuck to the glass and showed no tendency to escape from the dish. After irradiation, the larvae were moistened with Ringer's solution to free them and removed with a camel's hair brush to shell vials containing culture medium to complete development. This drying treatment had no deleterious effect on the larvae: several groups of larvae were handled in this way and dried 20 to 30 minutes without irradiation and all hatched out normally.

Results

Larvae show a gradual increase in sensitivity to ultraviolet radiation with age from 50 to 100 hours after hatching to a maximum at one hour after pupation, then a sharp decrease in sensitivity with pupal age. The sensitivity of flies to ultraviolet at an intensity of 44 ergs per mm.^{2} per second for different durations of exposure, as measured by the percentages killed, is given in Figure 1.

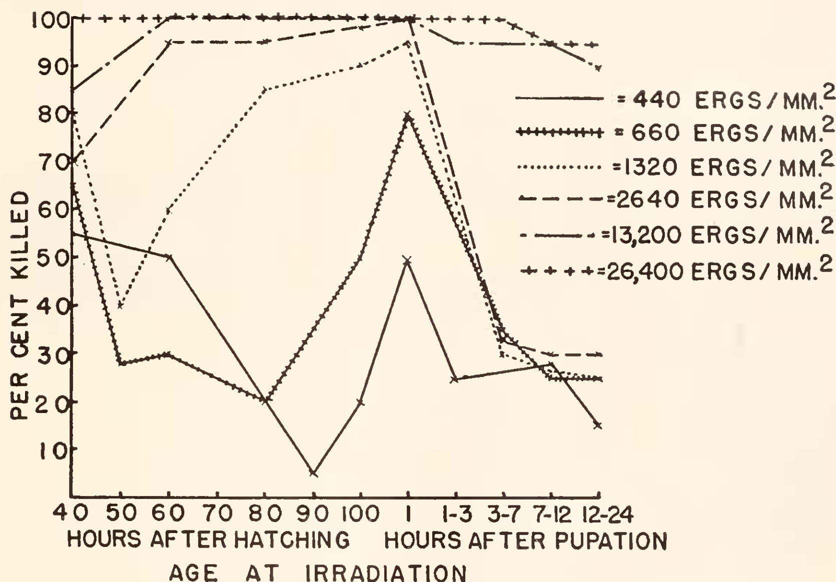

Figure 1. Percentage of flies killed by different total dosages of irradiation at different ages given at 44 ergs/mm.^{2}/sec.

The percentage of flies showing phenocopies also varies with the age of the larva or pupa irradiated (Fig. 2). There seem to be two periods during the larval stage of greater phenocopy production for a given amount of radiation, one about 50 and one about 100 hours after the larvae hatch from the egg. The entire larval period from 40 to 100 hours after hatching is one of fairly high phenocopy production, higher than the first 24 hours of pupal life. Some pupae showed very high percentages of phenocopies, up to 400 per cent (400 phenocopies per 100 flies), but this was caused by the fact that the pupae can withstand more energy for the production of phenocopies without being killed. The high percentages of phenocopies were the result of irradiations with 13,200 or 26,400 ergs/mm.^{2} Since these dosages killed 100 per cent of the flies irradiated at most of the ages used, no lines were drawn for them on Figure 2. In 7- to 12-hour pupae, irradiations of 3,960 ergs/mm.^{2} gave 120 phenocopies per 100 flies, 13,200 ergs/mm.^{2}, 200 phenocopies per 100 flies, and 26,400 ergs/mm.^{2}, 300 phenocopies per 100 flies.

The phenocopies produced included abnormal abdomen, combgap legs, abnormal thorax, small or rough eye, fused eye facets, folded, dumpy, curled or balloon wings, abnormalities in the wing veins, fused, singed or missing bristles and microchaetes,

doubled sex combs on a male prothoracic leg, and a shoulder-like protrusion growing anteriorly from the mesothorax.

The most numerous type of phenocopy produced was that involving an abnormality in the abdomen, some deformity in or the complete absence of one or more tergites. These were produced by irradiating flies in any stage of development from 40-hour larvae to 24-hour pupae. Geigy (1931) produced similar phenocopies by irradiating eggs ½ to 17½ hours old. (See Geigy for figures of the variations in phenotype produced.) Epsteins (1939) reported similar abnormali-

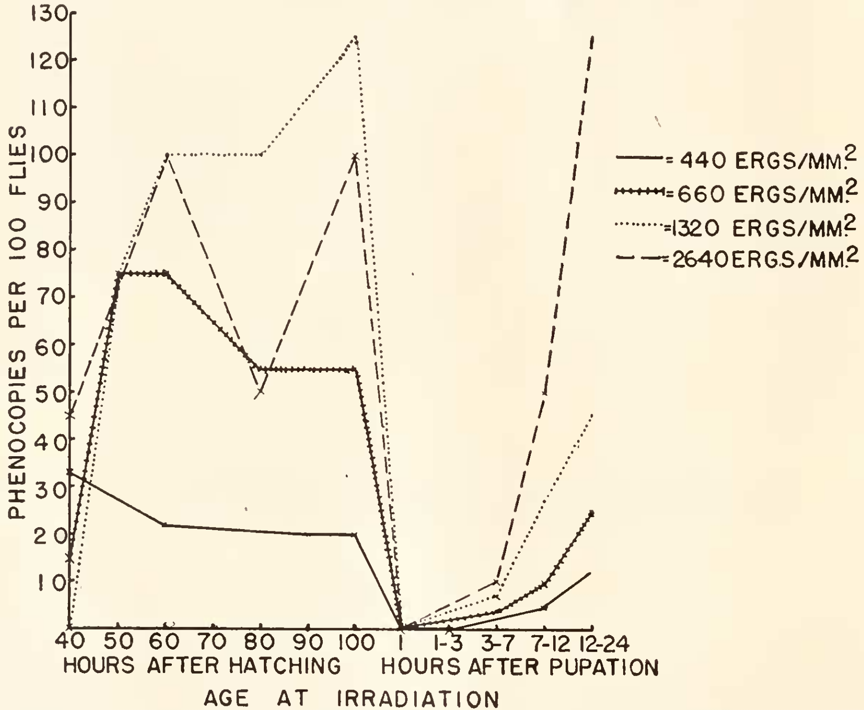

FIGURE 2. Number of phenocopies per 100 flies produced by different total dosages of irradiation at different ages given at 44 ergs/mm.^{2}/sec.

ties produced by irradiations at times comparable to those used in this study. The large numbers of these resulting from irradiations during either the egg, larval, or early pupal stage is probably due to the fact that the dorsal abdominal imaginal discs lie close to the dorsal surface and are more readily reached by the rays than are deeper-lying discs. Eloff (1939) showed that ultraviolet light penetrates one thickness (about 10 microns) of Drosophila cuticle with only slight diminution of effect, but that four thicknesses screen out the effective rays.

Abnormalities in the formation of the mesothorax and scutellum occur after irradiation during the pupal period, especially between 7 to 12 and 12 to 24 hours after pupation. These include absence of the scutellum, failure of the right and left dorsal mesothoracic discs to fuse, failure of one disc to unfold properly, etc., giving phenotypes similar to those of certain grades of tetraltera (see Villee, 1942,

for figures). The eye phenocopies, small, rough, or fused eye facets, also were obtained after irradiation in the pupal period, especially between 7 and 24 hours after pupation. One fly irradiated in this period showed large, swollen legs resembling the phenotype of combgap. The wing and bristle phenocopies were produced primarily by irradiation in the pupal period, 7–24 hours after pupation. Reduction of the microchaetes occurred only after irradiation in this period. An additional period in which both bristle and wing phenocopies were produced was between 80 and 90 hours of larval life. Dumpy wings were produced only by the irradiation of the pupae.

The three stocks used in general responded similarly to irradiations of comparable developmental stages. The single exception was aristapedia-Bridges, which frequently developed 2- to 4-segmented tarsi when irradiated as 80- or 90-hour larvae or as 7- to 12-hour pupae. This same stock also develops very short, 1- to 4-segmented tarsi when treated with X-rays (Villee, 1946a), low temperature (Villee, 1943) or when combined with "growth rate" genes (Villee, 1945), although under normal conditions it always has normal 5-segmented tarsi (see Villee, 1946a for discussion).

Only two phenocopies were found which resembled the pervasive effects of overgrowth or abnormal histogenesis found frequently with X-radiation of 70- to 90-hour larvae (Waddington, 1942; Villee, 1946a). One wild type male irradiated as a 3- to 7-hour pupa with a total of 5,280 ergs/mm.^{2} developed a second pair of sex combs on the second tarsal segment of the prothoracic leg, and resembled closely a phenotype obtained with X-radiation (Villee, 1946a, Fig. 8). One wild type female irradiated 100 hours after hatching from the egg, about 2 hours before pupation, with 2,640 ergs/mm.^{2} developed a small palp-like outgrowth from the anterior dorso-lateral margin of the thorax, apparently identical with those obtained with X-radiation (Villee, 1946a, Figs. 1–3).

Irradiation of larvae with ultraviolet does not cause a retardation of pupation as X-radiation does. The larvae pupate in the normal time after ultraviolet treatment; X-radiation causes a retardation of pupation of 8 to 12 days beyond the time when controls pupate (Villee, 1946). With X-radiation there is also a marked difference in the results of irradiation of about the same dosage given at different intensities. A given dosage at high intensity causes a much higher lethality than a similar dosage at low intensity. With ultraviolet radiation, this difference is not found. A given dosage of ultraviolet light, whether given at 44, 37, 27, 17.5, or 11 ergs/mm.^{2}/sec., produces about the same percentage of lethality and of phenocopies. It may be that the range of ultraviolet intensities used was not great enough. There is only a factor of four between the lowest and highest intensities, whereas in the X-ray experiments the high intensity was 71 times the low intensity (5,540 *vs.* 78 r. units per minute). However, Carlson and Hollaender (1944) found that the effects of 2537 A light on mitosis in grasshopper neuroblasts depend simply on the total dosage and not on the intensity even when it is varied by a factor of 1500. In a later paper (Carlson and Hollaender, 1945) they found that at low total dosages (57.6 ergs/mm.^{2}) intensities varying from 0.004 to 16.3 ergs/mm.^{2}/sec. showed no significant differences in the effect on mitosis, but at high total doses (172.8 and 230.4 ergs/mm.^{2}) treatments given at high intensity were slightly more effective in depressing mitosis than ones at low intensity. The dosages used in this study were higher than Carlson and Hollaender's highest but

no intensity effect was observed. Bain and Rusch (1943) reported just the opposite conditions in the production of tumors in mice by ultraviolet radiation. They found ultraviolet of wave-lengths 2800–3400 A more effective in producing tumors when given at low intensities over long periods of time than when given at high intensities for short periods. They used much greater amounts of energy, 116–212 $\times 10^5$ ergs/mm.^{2}, than used by Carlson and Hollaender in their experiments. The high intensity, 1.35 $\times 10^5$ ergs/mm.^{2}/day, was about four times their low intensity, 0.35 $\times 10^5$ ergs/mm.^{2}/day.

Discussion

The factors regulating the production of phenocopies by temperature treatments are: (1) the developmental stage at which the treatment is applied; (2) the extensity (total time) of the treatment; (3) the intensity of the treatment; and (4) the genotype of the animals treated (Goldschmidt, 1929, 1935). With ultraviolet light, the important factors are the age of the fly at irradiation, the stock used, and the total dosage of irradiation. Within the range of intensities used in these experiments, variations in intensity had no effect in changing the percentage of lethality or of phenocopies. The phenocopies produced may, in the main, be explained as due to the absorption of the light energy in individual cells or small groups of cells, probably by the nucleic acids, since 2537 A is close to the region in which they absorb maximally, 2600 A. Since both desoxyribose nucleic acid, located in the chromosomes, and ribose nucleic acid, located in the cytoplasm as well as in the chromosomes, absorb at the same wave-length, it is impossible to decide whether the phenocopy-producing reaction is localized in the nucleus, cytoplasm, or in both. The fact that ultraviolet of 2537 A wave-length affects the chromosomes and retards mitosis in grasshopper neuroblasts (Carlson and Hollaender, 1944) would suggest that the phenocopy effect is also mediated by the chromosomes. Hollaender, Greenstein, and Jenrette (1941) found that 2537 A radiation causes a depolymerization of sodium thymonucleate (desoxyribonucleate) *in vitro*. As a working hypothesis we may suppose that irradiation of Drosophila larvae or pupae with 2537 A is absorbed by nucleic acids or nucleoproteins in the chromosomes of the cells of the imaginal discs near the surface. The absorption of this energy results in a physical change, a depolymerization, of the nucleic acid with a consequent upset in the structure of the gene so that it is partially or completely inactivated, with the result that development of that structure is abnormal. Since the inactivated genes are located in some body cell rather than a germ cell, a phenocopy rather than a mutation is produced. Future research may, of course, show that the phenocopy-producing mechanism is entirely different from that proposed here.

Some of the phenocopies found in these experiments suggest that the cells of the imaginal discs were killed, others suggest that certain genes were altered or inactivated, perhaps by the scheme outlined above. Only two of the phenocopies, a doubling of the sex combs on the male prothoracic leg, and a palp-like outgrowth from the anterior dorso-lateral margin of the thorax, each of which occurred only once in the course of the study, suggest the pervasive effects of abnormal histogenesis found frequently with X-radiation. These have been explained (Waddington, 1942; Villee, 1946a) by assuming that the X-rays cause the death of cells

and that the dead or necrotic cells release diffusible morphogenetic substances which result in the abnormal histogenesis or overgrowth. The same explanation may be applied to the abnormal histogeneses produced by ultraviolet radiation.

The sensitive periods for certain of the phenocopies are slightly different from those found for X-rays or temperature treatments. It is rather difficult to compare the work of different investigators, who use different stocks raised at different temperatures, but it would appear that the sensitive period for the production of dumpy wings by ultraviolet corresponds with that found by Blanc and Child (1940) for temperature treatments and that the larval sensitive period for bristle reduction by ultraviolet is identical with the temperature sensitive period determined by Child (1935). In addition there is a sensitive period for bristle reduction by ultraviolet in the pupal stage which was also found in X-radiation experiments (Waddington, 1942; Villee, 1946a). The sensitive period for the reduction of the size of the eye by ultraviolet is in the pupal period, 7–24 hours after pupation, whereas Goldschmidt (1935) found the temperature sensitive period to be in the larval period, at an age corresponding to approximately 90 hours after hatching from the egg. Abnormalities in the thorax appear after ultraviolet irradiation in the pupal period, 7–24 hours after pupation, but in the larval period at ages corresponding to 50–100 hours after hatching from the egg following X-radiation (Villee, 1946a). The wing phenocopies produced by ultraviolet had two sensitive periods, one in the larval period about 80–90 hours after hatching from the egg, which corresponds to the temperature sensitive period (Goldschmidt, 1935) and an additional one in the pupal period not found with temperature treatments.

The energy threshold for the production of phenocopies is slightly below the lowest total dosages, 330 and 440 ergs/mm.^{2}, used in these experiments and varies with the age of the fly irradiated. At one hour after pupation, no phenocopies were produced by 440 ergs/mm.^{2} but the dosage caused a 50 per cent mortality among the pupae irradiated. At other ages, from 5 to 33 per cent phenocopies (i.e., 5 to 33 phenocopies per 100 flies) were obtained with this dosage. This threshold level is of the same order of magnitude as that found by Landen and Uber (1939) for the inactivation of yeast (500 ergs/mm.^{2} produced a 50 per cent inactivation) and by Giese (1946) for the production of abnormalities in developing echinoderms by the irradiation of sperm before fertilization. He found the threshold level for the production of abnormalities in development by irradiating the eggs of these forms to be considerably higher, on the order of 5,000–8,000 ergs/mm.^{2} Carlson and Hollaender (1945) found a considerably lower threshold in the effects of ultraviolet on mitosis in grasshopper neuroblasts: about 100 ergs/mm.^{2} produce a reduction of the mitotic ratio to 0.5. Davis (1944) found that the energy required to inhibit the folding process in neural tube formation varied with the wave-length of the ultraviolet used and that about 200 ergs/mm.^{2} produced the inhibition when ultraviolet of 2537 A was used.

It is impossible to make an exact comparison between the actions of X-rays and ultraviolet rays per energy unit, first, because their modes of action are different, and second, because only a small percentage of the X-rays are absorbed and become effective in the tissue irradiated, the rest pass through without affecting the cells, whereas ultraviolet rays are largely absorbed by tissues as thick as a Drosophila larva or pupa. However, it is of interest to make a rough comparison between the experiments reported here and the previous study using X-rays

(Villee, 1946a). It was found that the threshold for the production of phenocopies with X-rays was slightly below 1096 r. units. At that level from 0 to 40 per cent of the flies were killed and from 0 to 10 per cent showed phenocopies. This dosage D in roentgens may be converted to the density of absorbed energy, E, by the formula $E = 83\ D$ ergs/cm.^{3} (Cole, personal communication) to give 90,968 ergs/cm.^{3} The absorbed energy of the ultraviolet radiation, computed from the area and volume of the larva and the minimal threshold intensity of 440 ergs/mm.^{2} and assuming total absorbtion by the organism, is 440,000 ergs/mm.^{3} From this it can be seen that X-radiation is approximately five times as effective per energy unit as ultraviolet radiation of wave-length 2537 A in producing phenocopies. I want to thank Professor Kenneth S. Cole for his assistance in making these calculations.

Summary

1. Drosophila larvae, prepupae and pupae of various ages and genotypes were irradiated with ultraviolet of wave-length 2537 A and in dosages varying from 330 to 79,200 ergs/mm.^{2} The phenocopies produced varied with the age of the irradiated fly, the stock used, and the total dosages of the irradiation. Irradiation with ultraviolet does not cause a retardation of pupation as X-radiation does.

2. Larvae show a gradual increase in sensitivity to ultraviolet radiation with age from 50 to 100 hours after hatching from the egg to a maximum at one hour after pupation, then a sharp decrease in sensitivity with pupal age. There are two periods in the larval stage, one about 50 and one about 100 hours after hatching, of greater phenocopy production for a given amount of radiation. Irradiations during the first 24 hours of pupal life produce fewer phenocopies for a given amount of radiation than during the larval period.

3. The sensitive periods for the production of certain phenocopies by ultraviolet are compared with the sensitive periods for X-ray and temperature treatments. Some are identical, a few are different.

4. Irradiations of the same total dosage produce the same percentages of lethality and of phenocopies whether given at high or low intensities. The threshold level for the production of phenocopies varies with the age of the fly irradiated but is about 440 ergs/mm.^{2} A comparison is made of this threshold with the thresholds for the effect of ultraviolet on other biological systems and with the effect of X-rays on phenocopy production in Drosophila.

5. A hypothesis for the mechanism involved in the production of phenocopies by ultraviolet rays is discussed.

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